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(21) International Application Number: PCT/DK89/00088 (22) International Filing Date: 14 April 1989 (14.04.89) (30) Priority data: 2123/88 15 April 1988 (15.04.88) DK (71) Applicant (for all designated States except US): NOVO INDUSTRI A/S [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK). (72) Inventors; and (75) Inventors/Applicants (for US only): KIRK, Ole [DK/DK]; Stefansgade 38, DK-2200 Copenhagen N (DK). DAMHUS, Ture [DK/DK]; Livjærggade 43 st.tv., DK-2100 Copenhagen Ø (DK). GOTFREDSEN, Sven, Erik [DK/DK]; Smedegade 15 B, DK-3500 Værløse (DK). (74) Common Representative: NOVO INDUSTRI A/S; Patent Department, Novo Allé, DK-2880 Bagsværd (DK).		(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent), US. Published <i>With international search report.</i> <i>With a request for rectification under Rule 91.1(f).</i>
(54) Title: A DETERGENT ADDITIVE FOR BLEACHING FABRIC (57) Abstract A detergent additive for use in a process for bleaching stained fabric comprises an enzyme exhibiting peroxidase activity and optionally also hydrogen peroxide or a precursor thereof, e.g. a perborate or percarbonate. The detergent additive may further comprise an enzyme capable of generating hydrogen peroxide, e.g. an oxidase, and a substrate therefor.		

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A DETERGENT ADDITIVE FOR BLEACHING FABRICFIELD OF INVENTION

5

The present invention relates to a detergent additive comprising an enzyme system capable of exerting a bleaching effect on fabrics in the course of washing procedures, a detergent composition containing the
10 detergent additive as well as a process for bleaching fabrics by treating the fabrics with an enzyme exerting a bleaching effect.

BACKGROUND OF THE INVENTION

15

The use of bleaching agents in washing procedures and as constituents of detergent compositions is well known in the art. Thus, bleaching agents are incorporated in or sold as constituents of a major part of the commercially
20 available detergent compositions. Traditionally, important bleaching agents incorporated in detergent compositions are compounds which act as precursors of hydrogen peroxide formed in the course of the washing procedure. Perborates and percarbonates are the most
25 important examples of compounds which are employed as detergent additives and which exert a bleaching effect in this fashion. The detailed mechanism of perborate and percarbonate bleaching is not known at present, but it is generally assumed that hydrogen peroxide converts coloured
30 substances responsible for the staining of fabric into uncoloured materials by oxidation and that some oxidation of the coloured substances may also take place due to their direct interaction with perborate or percarbonate.

35 Although these traditional bleaching systems function quite satisfactorily in many instances and have become widely accepted, many attempts to improve their

performance have been made over the years. An important aim has been to provide bleaching systems which function at low temperatures and which, in general, are more efficient than the traditional systems. Also, it has been 5 an important aim to develop systems leading to a better utilization of a bleaching agent incorporated in detergent compositions or otherwise employed for the bleaching of fabrics. Among other things, these developments have been prompted by the wish of the consumer to conduct washing at 10 low temperatures which are not optimal for the function of the traditional bleaching systems, the optimum operation temperature of which is at least 60°C, and by the environmental aspects of releasing, to the environment, large quantities of bleaching agents added in excess 15 quantities to detergent compositions in order to obtain an adequate bleaching, but not utilized fully in the course of washing procedures.

In connection with these attempts to develop improved 20 bleaching systems considerable attention has been directed towards the possibility of generating powerful and effective bleaching species during washing by means of hydrogen peroxide from its traditional precursors such as perborate and percarbonate. An example of a successful 25 development along these lines is the TAED (tetraacetyl ethylene diamine) based system now used widely in detergent compositions (cf. for instance Second World Conference on Detergents, A.R. Baldwin (ed.), American Oil Chemists' Society, 1987, pp. 177-180.

30

SUMMARY OF THE INVENTION

It has surprisingly been found possible to enhance the bleaching effect of hydrogen peroxide during washing 35 procedures by employing a group of enzymes utilizing hydrogen peroxide as a substrate for the oxidation of

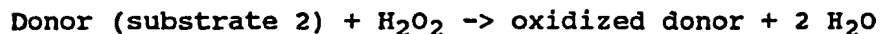
organic or inorganic substances (such enzymes are usually termed peroxidases).

Accordingly, the present invention relates to a detergent additive capable of exerting a bleaching effect on fabrics and comprising an enzyme exhibiting a peroxidase effect. The detergent additive of the invention will usually also comprise hydrogen peroxide or a precursor of hydrogen peroxide, preferably perborate or percarbonate. In the present context, the term "enzyme exhibiting a peroxidase effect" is understood to indicate an enzyme with a mode of action similar to that of peroxidase and will be used synonymously therewith.

It is well recognized in the art (cf. for instance B.C. Saunders et al., Peroxidase, London, 1964, p. 10 ff.) that peroxidases act on various amino and phenolic compounds resulting in the production of a colour. In view of this, it must be considered surprising that peroxidases may also exert a bleaching effect on coloured substances. While the mechanism governing the ability of peroxidases to effect bleaching of stains present on fabrics caused by such coloured substances has not yet been elucidated, it is currently believed that these enzymes act by reducing hydrogen peroxide (substrate 1) and oxidizing the coloured substance in the stain (substrate 2), thereby generating a colourless substance and consequently destaining of the fabric. This reaction is shown in Reaction Scheme 1 below

30

Reaction Scheme 1:



35

As the peroxidase will generally show affinity for coloured substances present in the stain which act as

substrates for the enzyme (these will typically be natural dyestuffs such as various polyphenols), bleaching will be targeted to the stains, for which reason a more efficient utilization of hydrogen peroxide is obtained by employing 5 peroxidase for bleaching than with the conventional bleaching methods where an excess of hydrogen peroxide must be present in order to achieve an adequate bleaching effect.

10 This implies that it may be possible, by using the detergent additives of the invention to bleach stains on fabric, to reduce the amount of hydrogen peroxide or precursors thereof in the additive or in detergent compositions containing the additive and yet provide a 15 satisfactory bleaching effect. Thus, the environmental load, i.e. the amount of unspent bleaching agent released into the environment, may be reduced when a detergent additive of the invention is employed for bleaching. Furthermore, as a smaller quantity of hydrogen peroxide 20 need be added to achieve efficient bleaching, the overall performance of detergent compositions in which such bleaching agents are included (the performance of, for instance, detergent enzymes tends to deteriorate when large amounts of bleaching agent are employed) may be 25 improved.

It is further contemplated that peroxidases with specificity for a particular class of colourant (e.g. betalaines, carotenoids, flavonoids, etc.) may be employed 30 for particular purposes, while a peroxidase of low specificity (i.e. acting on several natural colourants) may be used for a more unspecific enzymatic bleaching.

It has previously been reported that peroxidases may 35 decolourize certain pigments (cf. for instance W. Schreiber, Biochem. Biophys. Res. Commun. 63 (2), 1975, pp. 509-514, describing the degradation of 3-

hydroxyflavone by horseradish peroxidase; A. Ben Aziz, Phytochemistry 10, 1971, pp. 1445-1452, describing the bleaching of carotene by means of peroxidase; and B.P. Wasserman, J. Food Sci. 49, 1984, pp. 536-538, describing
5 the decolourization of betalain by horseradish peroxidase). These publications all describe test methods whereby the pigment in question is incubated with the enzyme in solution. Hence, they do not in any way indicate that peroxidases may be efficient for bleaching usually
10 dry stains in fabrics in which the coloured substance(s) may have become absorbed or partly absorbed into the fibers of the fabric so that they are less accessible to enzymatic action and/or in which the coloured substances may have been oxidized by atmospheric oxygen so that, in
15 effect, a different coloured substance than that present in a solution of the dye may have been produced.

Furthermore, Ben Aziz et al. and Wasserman et al. present the bleaching action of peroxidase on carotene and
20 betalain, respectively, as a problem when using these pigments as food colourants, which problem must be counteracted by including an antioxidant in the foodstuff in question. Thus, they do not consider the peroxidase-mediated bleaching of these pigments to have any practical
25 utility in itself.

Apart from this, it has been suggested to bleach paper pulp by means of peroxidases, cf. for instance US
4,690,895.

30

DETAILED DISCLOSURE OF THE INVENTION

Peroxidases which may be employed for the present purpose as detergent additives may be isolated from and are
35 producible by plants (e.g. horseradish peroxidase) or microorganisms such as basidiomycetes, fungi, actinomycetes, or bacteria. Some preferred microorganisms

include strains of Fusarium, in particular Fusarium oxysporum, strains of Streptomyces, in particular Streptomyces thermoviolaceus or Streptomyces viridosporus, strains of Pseudomonas, preferably Pseudomonas putida or
5 Pseudomonas fluorescens, strains of Coprinus, in particular Coprinus cinereus, Coprinus macrorhizus or Coprinus cinereus f. microsporus, strains of Streptovercillium, in particular Streptovercillium verticillium ssp. verticillium, strains of Bacillus, in
10 particular Bacillus stearothermophilus, and strains of Coriolus, preferably Coriolus versicolor, strains of Phanerochaete, in particular Phanerochaete chrysosporium.

Specific examples of preferred strains are Coprinus
15 cinereus f. microsporus IFO 8371, Coprinus cinereus IFO 30114, Pseudomonas fluorescens NRRL B-11, Streptovercillium verticillium ssp. verticillium IFO 13864, Streptomyces thermoviolaceus CBS 278.66, Streptomyces viridosporus, ATCC 39115, Streptomyces badius
20 ATCC 39117, Streptomyces phaeochromogenes NRRL B-3559, Pseudomonas pyrrocinia ATCC 15958, Fusarium oxysporum DSM 2672 and Bacillus stearothermophilus ATCC 12978.

Other potential sources of useful peroxidases are listed
25 in B.C. Saunders et al., op. cit., pp. 41-43.

Particularly preferred peroxidases are those which are active at the typical pH of washing liquors, i.e. at a pH of 6.5 - 10.5, preferably 6.5 - 9.5, and most preferably
30 7.5 - 9.5. More specifically, the peroxidases of interest for the present purpose are those which exhibit at least 25% of their optimal activity (i.e. their activity at the pH optimum) in these pH ranges, e.g. as determined by the ABTS assay described in Example 1 below. Such peroxidases
35 may be isolated by screening for peroxidase production by alkalophilic microorganisms, e.g. by the ABTS assay described in Example 1 below.

Other preferred peroxidases are those which exhibit a good thermostability, in particular those retaining at least 25% of their initial activity after 20 minutes at 40°C, as well as a good stability towards commonly used detergent components such as non-ionic, cationic, or anionic surfactants, detergent builders, phosphate etc., in particular those retaining at least 25% of their initial activity after exposure to such detergent ingredients for 20 minutes.

Methods of producing enzymes to be used according to the invention are described in the art, cf. for example FEBS Letters 1625, 173(1), Applied and Environmental Microbiology, Feb. 1985, pp. 273-278, Applied Microbiol. Biotechnol. 26, 1987, pp. 158-163, Biotechnology Letters 2(5), 1987, pp. 357-360, Nature 326, 2 April 1987, FEBS Letters 4270, 209(2), p. 321.

Another group of useful peroxidases is constituted by the ligninases: these enzymes which exert a strong peroxidase activity are instrumental in the degradation of lignin (e.g. in wood) and are produced by a variety of wood rot fungi. They have previously been suggested for the bleaching of paper pulp (cf. for instance US 4,690,895). Further useful peroxidases are haloperoxidases, such as chloro- and bromoperoxidases, as they are able to oxidize halide ions to hypohalites which are powerful bleaching agents, apart from being able to oxidize organic compounds.

The peroxidase may furthermore be one which is producible by a method comprising cultivating a host cell transformed with a recombinant DNA vector which carries a DNA sequence encoding said peroxidase as well as DNA sequences encoding functions permitting the expression of the DNA sequence encoding the peroxidase, in a culture medium under

conditions permitting the expression of the peroxidase and recovering the peroxidase from the culture.

A DNA fragment encoding the peroxidase may, for instance, be isolated by establishing a cDNA or genomic library of a peroxidase-producing microorganism, such as one of the organisms mentioned above, and screening for positive clones by conventional procedures such as by hybridization to oligonucleotide probes synthesized on the basis of the full or partial amino acid sequence of the peroxidase, or by selecting for clones expressing the appropriate enzyme activity, or by selecting for clones producing a protein which is reactive with an antibody against a native peroxidase.

15

Once selected, the DNA sequence may be inserted into a suitable replicable expression vector comprising appropriate promotor, operator and terminator sequences permitting the peroxidase to be expressed in a particular host organism, as well as an origin of replication enabling the vector to replicate in the host organism in question.

The resulting expression vector may then be transformed into a suitable host cell, such as a fungal cell, preferred examples of which are a species of Aspergillus, most preferably Aspergillus oryzae or Aspergillus niger. Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known per se. The use of Aspergillus as a host microorganism is described in EP 238,023 (of Novo Industri A/S), the contents of which are hereby incorporated by reference.

35

Alternatively, the host organisms may be a bacterium, in particular strains of Streptomyces and Bacillus, or E.

coli. The transformation of bacterial cells may be performed according to conventional methods, e.g. as described in T. Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, 1982.

5

The screening of appropriate DNA sequences and construction of vectors may also be carried out by standard procedures, cf. T. Maniatis et al., op. cit.

- 10 The medium used to cultivate the transformed host cells may be any conventional medium suitable for growing the host cells in question. The expressed peroxidase may conveniently be secreted into the culture medium and may be recovered therefrom by well-known procedures including
15 separating the cells from the medium by centrifugation or filtration, precipitating proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the
20 like.

Due to the possibility of reducing the quantity of hydrogen peroxide required for the bleaching of stains on fabrics by employing a peroxidase as described above, it
25 is possible to utilize an enzymatic process for hydrogen peroxide formation which has not previously had any technical interest due to difficulties in generating sufficient quantities of hydrogen peroxide. Thus, the detergent additive according to the invention may
30 additionally comprise a enzymatic system (i.e. an enzyme and a substrate therefor) which is capable of generating hydrogen peroxide.

One such category of hydrogen peroxide generating systems
35 comprises oxidases which are able to convert molecular oxygen and an organic or inorganic substrate into hydrogen peroxide and the oxidized substrate, respectively. In

themselves these enzymes are of limited or no use for bleaching stained fabric as they produce too small quantities of hydrogen peroxide, but they may be employed to great advantage in detergent additives of the invention 5 as the presence of peroxidase ensures an efficient utilization of the hydrogen peroxide produced, as indicated above.

Preferred oxidases are those which act on cheap and 10 readily available substrates which may conveniently be included into detergent compositions. An example of such a substrate is glucose which may be utilized for hydrogen peroxide production by means of glucose oxidase. Other suitable oxidases are urate oxidase, galactose oxidase, 15 alcohol oxidases, amine oxidases, amino acid oxidase and cholesterol oxidase.

The detergent additive of the invention may suitably be in the form of a non-dusting granulate, a liquid, in 20 particular a stabilized liquid, or a protected enzyme. Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 (both to Novo Industri A/S) and may optionally be coated by methods known in the art. Liquid enzyme preparations may, for instance, be 25 stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

30

The detergent additive may further include one or more other enzymes, such as protease, lipase or amylase, conventionally included in detergent additives.

35 In a still further aspect, the invention relates to a detergent composition comprising an enzyme exhibiting peroxidase activity as well as hydrogen peroxide or a

precursor thereof, preferably a perborate or percarbonate. These and other components essential and/or advantageous for obtaining a bleaching of stained fabric (e.g. an oxidase and a substrate therefor) may be added to the
5 detergent composition separately or may be included in the form of a detergent additive as described above.

Detergent compositions of the invention additionally comprise surfactants which may be of the anionic, non-
10 ionic, cationic, amphoteric, or zwitterionic type as well as mixtures of these surfactant classes. Typical examples of anionic surfactants are linear alkyl benzene sulfonates (LAS), alpha olefin sulfonates (AOS), alcohol ethoxy sulfates (AES) and alkali metal salts of natural fatty
15 acids.

Detergent compositions of the invention may contain other detergent ingredients known in the art as e.g. builders, anti-corrosion agents, sequestering agents, anti soil-
20 redeposition agents, perfumes, enzyme stabilizers, etc.

It is at present contemplated that the detergent composition of the invention may contain the peroxidase in an amount corresponding to 0.1-100 mg peroxidase per liter
25 of washing liquors.

The detergent composition of the invention may be formulated in any convenient form, e.g. as a powder or liquid. The enzyme may be stabilized in a liquid detergent
30 by inclusion of enzyme stabilizers as indicated above. Liquid detergents may further include stabilized hydrogen peroxide precursors. Usually, the pH of a solution of the detergent composition of the invention will be 7-12 and in some instances 7.0-10.5. Other detergent enzymes such as
35 proteases, lipases or amylases may be included the detergent compositions of the invention, either separately or in a combined additive as described above.

In a further aspect, the present invention relates to a process for bleaching fabric which comprises treating fabric with a detergent additive or detergent composition as described above. The invention also relates to a bleaching process wherein fabric is treated with an enzyme exhibiting peroxidase activity in the presence of hydrogen peroxide or a precursor thereof, optionally in the presence of an oxidase and a substrate for the oxidase. As indicated above, the process is particularly well suited for bleaching stains caused by natural coloured substances, e.g. polyphenols, found in, for instances, fruit juice, wine, tea, and the like. The bleaching treatment may suitably be conducted in a soaking, washing or rinsing process. It is contemplated that the detergent additive or detergent composition may also be employed for the bleaching of textiles, e.g. during their manufacture.

The present invention is further illustrated by the following examples which are not in any way intended to limit the scope of the invention.

Example 1:

25

Cotton swatches homogeneously soiled with unsweetened blackcurrant juice were subjected to a model washing treatment to evaluate the bleaching effect of the system horseradish peroxidase + hydrogen peroxide. At a level of 10 mmol l⁻¹ H₂O₂ (equivalent to ca. 1.6 g sodium perborate tetrahydrate per litre washing liquor), a dosage of 5 mg/l horseradish peroxidase (Boehringer-Mannheim) was found to be advantageous.

35 The experimental conditions were:

13

Washing liquor: 0.01 M borax, pH adjusted to 7.5 with
phosphoric acid
Duration of washing
treatment: 30 min.
5 Temperature: 25°C
Textile/liquor ratio: 10 g/l

Evaluation of bleaching effect: The washed swatches were
10 rinsed in demineralized water, then dried overnight in the
dark, after which remission in the wavelength range 400-
700 nm was measured on a Datacolor Elrephometer 2000.

The table below gives relative detergency values obtained,
15 the detergency function itself being defined by

$$20 \text{ detergency} = \frac{R(\text{soiled, washed}) - R(\text{soiled, not washed})}{R(\text{unsoiled, not washed}) - R(\text{soiled, not washed})}$$

with R denoting remission. The reference treatment was
25 with borax solution as above, but without any bleach. The
results for a treatment with 10 mmol l⁻¹ H₂O₂ without
peroxidase are shown by way of comparison.

	Wavelength (nm)	delta-detergency with respect to no-bleach-wash (%)	
		H ₂ O ₂ alone	H ₂ O ₂ + peroxidase
5	400	1.2	3.3
	420	1.4	3.4
	440	1.8	3.4
	460	2.3	3.6
	480	2.5	3.6
10	500	2.6	3.5
	520	2.7	3.6
	540	2.9	3.9
	560	3.4	4.5
	580	4.0	5.4
15	600	5.3	7.3
	620	7.8	10.6
	640	10.6	14.7
	660	13.6	18.7
	680	15.4	20.3
20	700	17.4	23.0

25 Relative enzyme activity was assayed before and after the washing experiment by a standard technique (described by R.E. Childs and W.G. Bardsley, Biochem. J. **145**, 1975, pp. 93-103) involving H₂O₂ oxidation of ABTS at pH 7.5, monitored at 418 nm (ABTS = 2,2'-azino-bis(3-ethylbenzthiazol-30 line-6-sulphonic acid), supplied by Boehringer-Mannheim as the diammonium salt). After washing the activity was 70% of the initial activity.

Example 2

Cotton swatches homogeneously soiled with unsweetened blackcurrant juice, red wine and tea were subjected to a 5 model washing treatment to evaluate the bleaching effect of the horseradish peroxidase/H₂O₂ system.

The experimental conditions were :

10 Washing liquor : 0.05 M sodium phosphate buffer with pH = 7.5 prepared with water of a hardness equivalent to 1.6 mM Ca²⁺.

Duration of wash : 60 min.

15

Temperature : 24°C.

Bleaching agent : H₂O₂, dosed to 10 mM.

20 Enzyme : Sigma P-8125 horseradish peroxidase, dosed to 2 mg/l.

Textile/liquor ratio: 10 g/l.

25 Evaluation of bleaching effect : determined as described in Example 1, except that tap water was used to rinse the swatches.

At 460 nm, the detergency values for treatment with enzyme 30 + H₂O₂ and treatment with only H₂O₂ were found to be :

16

	Detergency	
	(enzyme + H ₂ O ₂)	(H ₂ O ₂ alone)
5		
Blackcurrant	40.2%	36.8%
Red wine	31.1%	27.3%
Tea	29.7%	24.6%
Unsoiled	80.6%	75.2%
10		

(The three types of swatches were washed together and were accompanied by unsoiled cotton swatches. The last line of the table concerns the unsoiled swatches.)

15

Similar experiments were conducted at a pH of 8.3 and at both pH values in the presence of an anionic surfactant (linear alkyl benzene sulfonate) and a non-ionic surfactant. None of these changes influenced the bleaching effect significantly, and the activity of the enzyme in the wash solution, as monitored by the ABTS assay described in Example 1, was essentially the same in all experiments (a relative activity of +/- 30%).

25 Example 3

Cotton swatches homogeneously soiled with beetroot juice were subjected several model washing treatments to evaluate the bleaching effect of the system H₂O₂ + peroxidase derived from Coprinus macrorhizus + H₂O₂.

The experimental conditions were :

Washing liquor : Potassium phosphate buffer 0.05 M
(see further below), with pH adjusted to 7.0.

17

Duration of wash : Varying, as specified below.

Temperature : 24°C.

5 Textile/liquor ratio: 11 g/l.

Hydrogen peroxide : 0.2 mM.

Enzyme : 20 mg/l peroxidase derived from
10 Coprinus macrorhizus (Chemical
Dynamics Corporation, New Jersey,
U.S., prod. no. 70-9590-00).

Evaluation of bleaching effect : determined as described
15 in Example 2.

The results were as follows :

20	Detergency				
		(enzyme + H ₂ O ₂)		(H ₂ O ₂ alone)	
		460 nm	600 nm	460 nm	600 nm
25	(1) Wash 70 min	75.2%	81.9%	75.9%	75.7%
	(2) Wash 15 min	61.8%	72.6%	57.9%	68.3%
	(3) Wash 1 min	48.5%	63.3%	45.2%	59.4%
	(4) Wash 1 min	51.0%	63.6%	48.8%	57.2%
30					

In experiment (1), the wash was followed by soaking over-
night. In experiments (2) and (3), the buffer solution was
prepared with water with a hardness equivalent to 1.6 mM
35 Ca²⁺, in (1) and (4) the buffer was made with demineral-
ized water.

SUBSTITUTE SHEET
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CLAIMS

1. A detergent additive capable of exerting a bleaching effect on fabrics and comprising an enzyme exhibiting peroxidase activity.
2. A detergent additive according to claim 1, which additionally comprises hydrogen peroxide or a precursor of hydrogen peroxide, preferably a perborate or percarbonate.
3. A detergent additive according to claim 1, wherein the peroxidase is one producible by a microorganism, e.g. a bacterium, fungus, actinomycete or basidiomycete.
4. A detergent additive according to claim 1, wherein the peroxidase is of plant origin.
5. A detergent additive according to claim 1, wherein the peroxidase is one producible by a method comprising cultivating a host cell transformed with a recombinant DNA vector carrying a DNA sequence encoding said peroxidase as well as DNA sequences encoding functions permitting the expression of the peroxidase, in a culture medium under conditions permitting the expression of the peroxidase and recovering the peroxidase from the culture.
6. A detergent additive according to any of the preceding claims, wherein the peroxidase is a ligninase.
7. A detergent additive according to any of claims 1-5, wherein the peroxidase is a haloperoxidase, such as a chloro or bromo peroxidase.
8. A detergent additive according to any of claims 1-6, wherein the peroxidase is active at a pH of 6.5-10.5, preferably 6.5-9.5, and most preferably 7.5-9.5.

9. A detergent additive according to any of claims 1-8, which additionally comprises an enzymatic system capable of generating hydrogen peroxide.
- 5 10. A detergent additive according to claim 10, wherein the enzymatic system comprises an oxidase.
11. A detergent additive according to claim 11, wherein the oxidase is selected from the group consisting of glucose
10 oxidase, urate oxidase, galactose oxidase, alcohol oxidases, amine oxidases, amino acid oxidase and cholesterol oxidase.
12. A detergent additive according to any of the preceding
15 claims, which additionally comprises one or more enzymes, in particular a lipase, protease, amylase or cellulase.
13. A detergent additive according to any of the preceding claims, which is in the form of a non-dusting granulate,
20 a liquid, in particular a stabilized liquid, or a protected enzyme.
14. A detergent composition which comprises a detergent additive according to any of claims 1-13.
- 25 15. A detergent composition which comprises an enzyme exhibiting peroxidase activity as well as hydrogen peroxide or a precursor for hydrogen peroxide, preferably a perborate or percarbonate.
- 30 16. A detergent composition according to claim 15, which further comprises an enzyme capable of generating hydrogen peroxide as well as a suitable substrate therefor.
- 35 17. A detergent composition according to claim 16, wherein the enzyme capable of generating hydrogen peroxide is an oxidase.

18. A process for bleaching fabric comprising treating fabric with a detergent additive according to any of claims 1-13 or a detergent composition according to claim 14 or
5 15.

19. A process according to claim 18 wherein the treatment of the fabric is conducted in a soaking, washing or rinsing process.

10

20. A process for bleaching fabric comprising treating fabric with an enzyme exhibiting peroxidase activity in the presence of hydrogen peroxide or a precursor of hydrogen peroxide.

15

21. A process according to claim 20, wherein said treatment is conducted in the presence of an oxidase and a substrate for said oxidase.

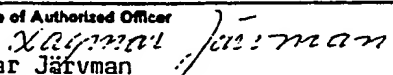
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REQUEST FOR RECTIFICATION PUBLISHED UNDER THE PROVISIONS
OF PCT RULE 91.1 (I)

Applicant hereby requests permission, pursuant to Rule 91.1 PCT, to rectify the obvious error made in omitting pages 8 and 13 from the description and enclose pages 8 and 13 for insertion into the description.

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK89/00088

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁴		
According to International Patent Classification (IPC) or to both National Classification and IPC ⁴		
C 11 D 3/395, 3/386		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC 4	C 11 D 3/39, 3/395, 3/386	
US C1	252: 174.12	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
SE, NO, DK, FI classes as above		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹		
Category ⁹	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X, Y	EP, A3, 0 173 378 (UNILEVER NV och UNILEVER PLC) 5 March 1986 see the abstract; page 2, lines 13-16 and claim 2 & JP, 61092569	1,5,7,14
A	US, A, 4 421 668 (R.B. COX ET AL.) 20 December 1983 see the claims and column 11, lines 10-20	1,14,18
Y	Chemical Abstracts, Vol. 101, no. 4, 23 July 1984, page 102, no. 25407e, Columbus, Ohio, US; Kasei K.K.: "Sprayable foaming cleaning compositions" & Jpn. Kokai Tokkyo Koho JP 58,191,800 /83,191,800/.	1,2,14,15
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
1989-07-17	1989-07-20	
International Searching Authority	Signature of Authorized Officer	
Swedish Patent Office	 Dagmar Järvman	